Probing the physiological function of ferredoxin-9 (Fx9) in *Synechocystis* sp. PCC 6803

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1. Introduction

Ferredoxins (Fx) can be found in most organisms and are probably among the oldest proteins on Earth[1]. They are small iron-sulfur cluster containing proteins that transfer electrons in a multitude of metabolic reactions, such as CO_2 fixation, nitrogen assimilation and hydrogen production[2,3]. The cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) contains at least nine isoforms with different types of iron-sulfur cluster[3]. The plant-like Fx1 (encoded by *ssl0020*) is the most abundant protein and is involved in almost all of the important metabolic pathways under photoautotrophic conditions. High *fx1* transcript levels have been observed after light induction[4]. Distinctive functions for all other ferredoxins remain largely unknown[2].

2. Construction of ferredoxin mutants

We generated several *Synechocystis* ferredoxin mutants (*fx2/Δfx2*, *Δfx3*, *Δfx4*, *fx5/Δfx5*, *Δfx6*, *Δfx7*, *fx8/Δfx8*, *Δfx9*) and a flavodoxin mutant (*ΔisiB*). The mutants were confirmed via PCR and DNA-sequencing (Table 1). It is worthy of note that the *fx2/Δfx2*, *fx5/Δfx5* and *fx8/Δfx8* mutants did not segregate, which indicates that these genes are essential for *Synechocystis*.

Furthermore, two double mutants (*Δfx7Δfx9* and *Δfx9ΔisiB*) and a triple mutant (*Δfx7Δfx8Δfx9*) were constructed from *Δfx9* (Table 1). All of these mutants are capable of growing under photoautotrophic condition on agar plates containing the corresponding antibiotics.

Table 1. Characteristics of the Fx-encoding genes in Synechocystis.

Name	Gene ID	Type of Iron Sulfur Center	Importance for Photo-Autotrophic Growth
fx1	ssl0020	[2Fe-2S] plant-like	Essential
fx2	sll1382	[2Fe-2S] plant-like	Essential
fx3	slr1828	[2Fe-2S] plant-like	Dispensable
fx4	slr0150	[2Fe-2S] plant-like	Dispensable
fx5	slr0148	[2Fe-2S] adrenodoxin-like	Essential
fx6	ssl2559	[2Fe-2S] plant-like	Dispensable
fx7	sll0662	[4Fe-4S] bacterial type	Dispensable
fx8	ssr3184	[3Fe-4S] [4Fe-4S] bacterial type	Essential
fx9	slr2059	[4Fe-4S] [4Fe-4S] bacterial type	Dispensable
isiB	sll0284	flavodoxin	Dispensable

3. Characterization of the Fx9-deletion mutant under LAHG conditions.

Previous work on *Chlamydomonas reinhardtii* ferredoxin-5 revealed that the respective mutant cannot grow in the dark, while its growth in the light was unaffected[5]. To date a similar phenotype has not been reported for a *Synechocystis* ferredoxin deletion mutant. However, in order to assess the function of *Synechocystis* ferredoxins under light-activated heterotrophic growth (LAHG) conditions, all of the ferredoxin deletion mutants were cultivated in the dark with 10 mM glucose as external carbon source and continuous air bubbling.

Fig. 1A shows that *Δfx9* and the respective double and triple mutants could not grow under LAHG conditions. However, the replacement of nitrate/nitrite with ammonium as the sole N-source rescued this growth phenotype (Fig. 1B). When grown on nitrite, the deletion of *fx9* inhibited nitrite consumption, but glucose uptake was unimpaired (Fig. 1C and 1D), which is likely used for glycogen accumulation. The higher concentration of excreted nitrite in the supernatant of *Δfx9* cultures indicated that the deletion of *fx9* might affect nitrate reduction (Fig. 1E). While *Δfx9* cannot grow on urea instead of nitrate under LAHG conditions (Fig. 1F), urea can be taken up and directly converted to ammonium by *Synechocystis* urease. Together these results indicate that Fx9 might not be involved in the reduction of nitrate to ammonium under LAHG growth conditions.

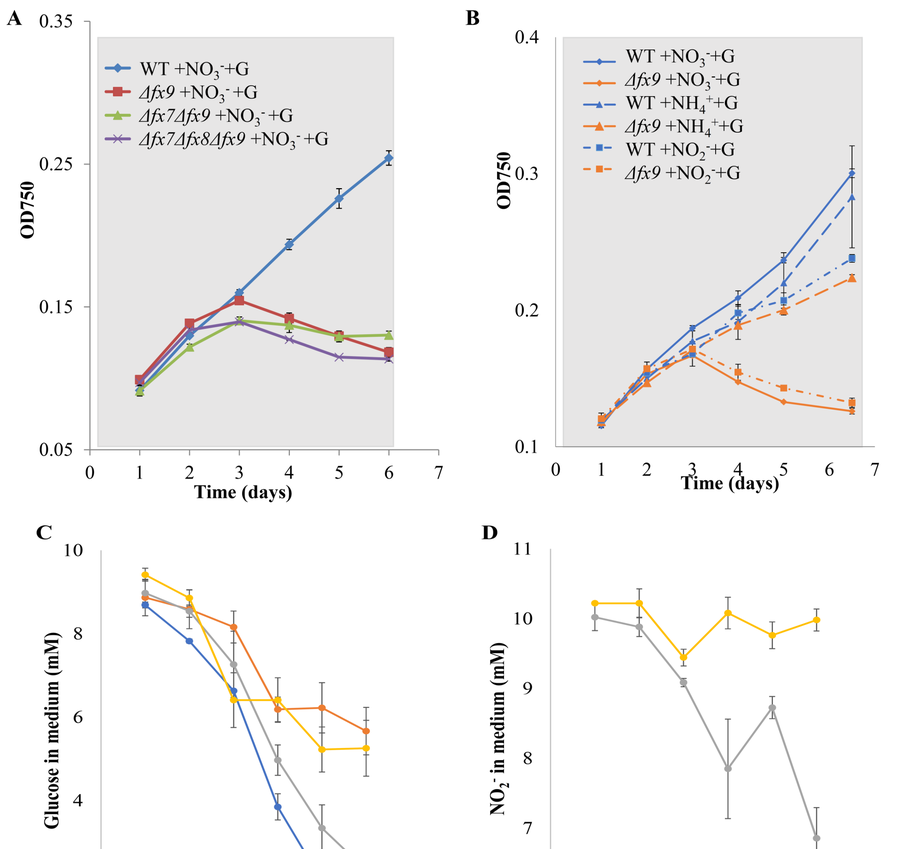

Fig. 1. A-B and F: Growth of ferredoxin deletion mutant with different N-source (nitrate: NO_3^-; nitrite: NO_2^-; ammonium: NH_4^+; Urea: U) under LAHG conditions. C-E: The carbon and nitrogen metabolism analysis of Δfx9 grown on either nitrate or nitrite under LAHG conditions.

4. Characterization of the Fx9-deletion mutant strain grown with day and night cycles.

When grown under autotrophic or mixotrophic conditions in a 12h day/ 12h night cycle, a condition resembling the natural light cycle, the deletion of *fx9* repressed cell growth (Fig. 2), indicating the physiological importance of Fx9 in *Synechocystis*.

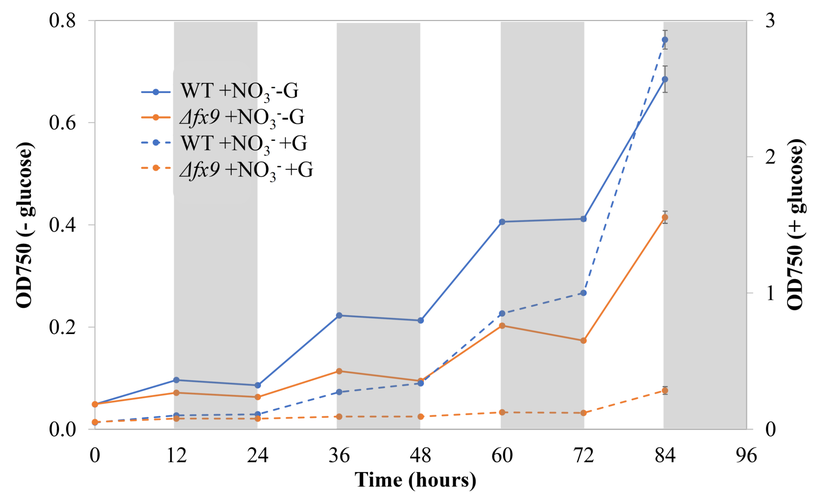

Fig. 2. Growth of Δfx9 under autotrophic and mixotrophic conditions with a 12h day/12h night cycle.

5. Conclusion and Outlook

- We deleted 8 of 9 ferredoxin and the flavodoxin genes in *Synechocystis*.
- The generated mutants were investigated under different growth conditions.
- Fx9 is essential for *Synechocystis* when grown under LAHG conditions and might be involved in pathways competing for electrons with the reduction of nitrate to ammonium.
- In the future we aim to identify potential Fx9 interaction partners.

References

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Acknowledgement

This work was supported by Deutsche Bundesstiftung Umwelt, Bundesministerium für Bildung und Forschung (BMBF) and China Scholarship Council (CSC).